Loss of Insulin-Like Growth Factor-II Imprinting and the Presence of Screen-Detected Colorectal Adenomas in Women

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Loss of imprinting (LOI) of insulin-like growth factor-II (IGF-II) may be an inherited epigenetic trait that is polymorphic in the population, and its presence may predispose an individual to the development of colorectal cancer. We evaluated the association between LOI of IGF-II in normal colonic mucosal samples and adenomas in women participating in a colonoscopy screening study. Among 40 participants, 11 (27.5%) had LOI of IGF-II in their normal colonic mucosal tissue. After adjusting for body mass index and family history of colorectal cancer, LOI status was associated with a fivefold increased risk of adenoma formation (odds ratio = 5.2, 95% confidence interval = 1.0 to 26.7). On average, IGF-II expression was more than threefold higher among women with LOI of IGF-II than among women with normal imprinting status. Our findings support the hypothesis that LOI of IGF-II is an epigenetic trait polymorphic in the population and suggest that LOI of IGF-II may play a role in colorectal cancer. These findings are intriguing and need to be confirmed in larger studies. [J Natl Cancer Inst 2004;96: 407–10]

Loss of imprinting (LOI) of insulin-like growth factor-II (IGF-II) is an epigenetic change that occurs in a variety of inherited and somatic cancers. LOI refers to the aberrant expression of both maternal and paternal alleles (or biallelic expression) when under normal conditions only one allele is expressed *(1–3)*. LOI of IGF-II may result in the overexpression of IGF-II, a mitogenic factor that promotes cell survival. The regulation of imprinting is thought to occur through differential DNA methylation between parental alleles, termed differential methylation regions. Studies *(4,5)* have described abnormal methylation patterns in these regions in tumors with LOI of IGF-II. Hypomethylation of the differential methylation region of IGF-II in both colonic tissue and lymphocyte DNA was correlated with LOI.

Cui et al. *(6)* observed LOI of IGF-II in 44% (12 of 27) of colon tumors but in only 13% (2 of 16) of colonic mucosa samples from patients undergoing surgery for diseases other than colon cancer. Cui et al. *(6)* also showed that patients with colon cancer who had LOI in their tumors also had LOI in their normal colonic mucosa and in their peripheral blood lymphocytes. In a subsequent study *(7)*, Cui et al. found that the odds of LOI of IGF-II in peripheral lymphocytes in patients undergoing colonoscopy with a past or present adenoma was 3.5 times and those with past or present colorectal cancer was 21.7 times that of patients without history of colorectal cancer or neoplasia. These findings suggest that LOI of IGF-II may be an epigenetic trait that is polymorphic in the population and that LOI of IGF-II in the colon may predispose individuals to develop colon cancer.

We evaluated LOI of IGF-II in normal colonic mucosal biopsy specimens among participants in an institutionally reviewed colonoscopy screening study (CONCeRN trial). From 1999 through 2002, women aged 50–79 years who were without symptoms of colorectal cancer and of average risk for the disease and women aged 40–79 years who were without symptoms but who had a family history of colorectal cancer were enrolled in the trial. Supplemental data at the Journal's Web site (http://jncicancerspectrum.oupjournals.org/jnci/content/vol96/issue5/) contains information regarding the CONCeRN trial. Participants were enrolled at regional military centers and excluded from the study if they had a personal history of adenomas, colorectal cancer, inflammatory bowel disease, hereditary non-polyposis colorectal cancer syn-

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drome, or familial adenomatous polyposis. All participants enrolled in the trial underwent a standard bowel preparation and colonoscopy, both of which were performed under the direct supervision of a staff gastroenterologist. Any visible polyps found during the colonoscopy were removed and their location described, i.e., cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid colon, or rectum. Board-certified pathologists at each regional medical center reviewed all excised polyps histologically. At the time of colonoscopy, six distal colonic mucosal biopsy specimens were removed from each participant and immediately snap-frozen.

Of the 1462 women eligible and enrolled in the trial, 299 (20%) had adenomas. For the LOI analysis, we selected a random sample of 53 women with prevalent adenomas and 53 age-matched women without evidence of adenomas. Lymphocyte DNA samples from the participants were genotyped for two separate IGF-II polymorphisms (820G/A and 266C/T) to identify informative subjects for LOI analysis (n = 48 participants heterozygous for either the 820 or 266 polymorphism). Supplemental data at the Journal's Web site contains a description of the specific genotyping assays. For each of the 48 informative participants, RNA was extracted from two mucosal biopsy specimens, DNase treated, and reverse transcribed into cDNA. RNA of sufficient quality was obtained from 40 participants who constituted our final study sample.

Total IGF-II and IGF-II 820G/A and 266C/T allele-specific gene expression assays were performed using real-time polymerase chain reaction (PCR) techniques based on Taqman Chemistry (PE Applied Biosystems, Foster City, CA). For each assay, the same forward and reverse PCR primers and two hybridization probes (each specific for one allele) were used. Supplemental data at the Journal's Web site contains the sequences of primers, hybridization probes, PCR conditions, and an example of a real-time amplification plot. β-actin expression was used as the internal reference standard and was evaluated using a predesigned Taqman assay (PE Applied Biosystems). Samples were quantified for total IGF-II cDNA expression by using a relative standard curve of known input cDNA concentration for total IGF-II and β-actin and by normalizing the levels of IGF-II expression to those of the β-actin internal control.

To investigate whether the assay could detect the relative contributions of the two alleles, we tested serial dilutions of DNA from individuals who were homozygous for either allele. The dilutions tested were 820G/A and 266C/T ratios as follows: 1:1, 3:1, 6:1, 9:1, 1:3, 1:6, and 1:9. DNA samples from individuals who were homozygous were run as controls. The relative level of allelic expression was determined by computing the differences in Ct scores—the number of PCR cycles it takes to reach a threshold of fluorescence—between the two alleles. For example, the differences in Ct scores for the IGF-II 820 G and A alleles were 1.2, 4.9, and 8.2, for dilutions of 1:1, 3:1, and 6:1, respectively. The coefficient of variation across dilutions averaged approximately 5%. Consistent with previous publications *(6,7)*, samples with a less than threefold difference in expression between the two alleles were considered to have LOI (i.e., biallelic expression). All samples were tested at least twice.

Characteristics of study participants (n = 40) according to LOI status are presented in Table 1. Eleven (27.5%) participants had LOI and 29 (72.5%) participants had normal allele-specific expression. Seventeen (43%) participants had one or more prevalent adenomas, and 23 (57%) participants were adenoma-free at colonoscopy. IGF-II LOI status in colonic mucosal samples from patients with and without prevalent adenomas is presented in Table 2. IGF-II LOI was observed in 41% of participants with adenomas compared with only 17% of participants without adenomas ($P = .09$). The odds ratio for adenoma prevalence after adjusting for body mass index and family history of colorectal cancer or adenomas was 5.2 (95% confidence interval = 1.0 to 26.7). Because LOI may be associated with risk of colorectal cancer and adenoma formation as a consequence of the expression of IGF-II from both alleles, we evaluated total IGF-II expression according to imprinting status and adenoma status (Table 3). IGF-II expression levels were, on average, more than threefold higher in participants with IGF-II LOI than in participants with normal imprinting status ($P = .10$). In addition, IGF-II expression levels were more than twofold higher in participants with adenomas than in patients without adenomas ($P = .10$).

Our data on LOI in mucosal samples are consistent with the data from Cui et al. *(7)* regarding LOI in peripheral blood

Table 1. Patient characteristics according to insulin-like growth factor-II (IGF-II) imprinting status in normal colonic mucosal samples among women patients of the CONCeRN Study*

Patient characteristic	All patients	Imprinting status LOI (n = 11)	Normal imprinting (n = 29)	P†
Mean age, y (SD)	60.2 (7.4)	58.6 (7.4)	60.9 (7.4)	.39
Mean weight, lbs (SD)	163.1 (35.7)	173.5 (49.6)	159.5 (29.8)	.30
Mean height, in (SD)	64.2 (3.0)	65.5 (2.4)	63.7 (2.5)	.10
BMI, mean (SD)‡	28.0 (6.6)	28.6 (9.4)	27.8 (5.5)	.73
% of smokers (SD)	14 (35.0)	3 (27.2)	11 (37.9)	.53
% of alcohol users (SD)	13 (32.5)	4 (36.4)	9 (31.0)	.75
% with family history of colorectal cancer (SD)	8 (20.0)	3 (27.2)	5 (17.2)	.48

*Details of the CONCeRN Study can be found at http://jncicancerspectrum.oupjournals.org/jnci/content/vol96/issue5/. LOI = loss of imprinting; SD = standard deviation; BMI = body mass index.

†*P* values for continuous variables are derived from Student's *t* test, and those for categorical variables were derived from the chi-square test. All *P* values were obtained from two-tailed tests, and *P* values less than .05 were considered statistically significant.

‡Body mass index was computed as weight in kg/height squared in meters.

Table 2. Association of loss of imprinting (LOI) of insulin-like growth factor-II (IGF-II) in colonic mucosal samples with adenoma formation among women patients of the CONCeRN Study*

Imprinting status	No. of patients (%) (n = 40)	Patients with adenomas (%) (n = 17)	Patients without adenomas (%) (n = 23)	OR (95% CI)
Normal	29 (72.5)	10 (58.8)	19 (82.6)	Referent
LOI	11 (27.5)	7 (41.2)	4 (17.4)	3.5 (0.8 to 14.1)† 5.2 (1.0 to 26.7)‡

*Details of the CONCeRN Study can be found at http://jncicancerspectrum.oupjournals.org/jnci/content/vol96/issue5/. Imprinting status was determined using real-time polymerase chain reaction techniques that were based on Taqman chemistry. Samples with a less than threefold difference in expression between the two alleles were considered to have LOI. OR = odds ratio; CI = confidence interval.

†OR and 95% CI for univariate estimate were determined using logistic regression.

‡OR and 95% CI from logistic regression model after adjusting for body mass index and family history of colorectal cancer or adenomas.

lymphocytes. We could not evaluate LOI status in peripheral blood lymphocytes because we were unable to obtain sufficient quality RNA from blood samples. Sakatani et al. *(8)* demonstrated LOI of IGF-II in peripheral blood lymphocytes in approximately 10% of disease-free individuals from Japan. LOI was also observed in these same individuals for another gene, imprinted multimembrane-spanning polyspecific transporter-like gene 1 (IMPT1) located on chromosome 11p15.5, but not for small nuclear ribonucleoprotein N (SNRPN), an imprinted gene located on chromosome 15q11-q13 *(9)*. Together these data suggest that LOI may not be specific for IGF-II but could affect a cluster of other imprinted genes located at 11p15.5. Thus, the etiologic role for LOI of IGF-II remains uncertain.

Table 3. Insulin-like growth factor-II (IGF-II) gene expression according to imprinting and adenoma status among women patients of the CONCeRN Study*

	Mean overall IGF-II expression (SD)†	*P*‡
Imprinting status		
Normal (n = 29)	0.31 (0.36)	.10
LOI (n = 11)	0.95 (1.15)	
Adenoma status		
Without adenomas (n = 23)	0.30 (0.32)	.10
With adenomas (n = 17)	0.74 (1.00)	

*Details of the CONCeRN Study can be found at http://jncicancerspectrum.oupjournals.org/jnci/content/vol96/issue5/. Imprinting status was determined using real-time polymerase chain reaction techniques that were based on Taqman chemistry. Samples with a less than threefold difference in expression between the two alleles were considered to have loss of imprinting (LOI). SD = standard deviation.

†Standardized ratio of expression of overall IGF-II expression relative to β-actin levels.

‡*P* values were derived from Student's *t* test, which allows for unequal variance for differences in means. All *P* values were obtained from two-tailed tests, and *P* values less than .05 were considered statistically significant.

LOI of IGF-II, studied frequently in cancer, has been reported in more than 20 different adult tumor types *(9)*. However, in some cancers, such as those of the breast, lung, and esophagus, LOI of IGF-II and other genes was shown to be specific to the tumor and was not found in the normal adjacent tissues *(10–12)*, suggesting that LOI may be a tumor-specific event and a predisposing factor only for certain cancer types. It is unclear whether LOI of certain genes is associated with the development of specific cancers or whether LOI is a general phenomenon associated with cancer. An assessment of the imprinting status of additional genes in normal colonic tissue and peripheral blood cells, of genes within the 11p15.5 cluster, and of genes positioned on other chromosomes may shed light on the role of imprinting abnormalities in the development of cancer.

The strengths of our study were the use of refined Taqman allelic discrimination assays to quantify allele-specific IGF-II expression and the conduct of the study within a colonoscopy screening trial with complete data on adenoma prevalence and patient characteristics. However, a limitation of our study was the modest sample size, which did not allow for an examination of LOI according to adenoma characteristics (e.g., advanced adenomas, multiple adenomas) but did contribute to the wide confidence interval we observed for the overall association.

We were able to quantify the level of total IGF-II expression present in the mucosal biopsy specimens. We found that, on average, mean levels of IGF-II expression were approximately threefold higher in specimens with LOI than in specimens with normal imprinting. Consistent with our findings, Ravenel et al. *(13)* assessed LOI of IGF-II in Wilms' tumors and found that IGF-II expression was increased 2.2-fold in tumors with LOI of IGF-II compared with tumors with normal imprinting. In another study *(14)*, however, IGF-II was overexpressed in Wilms' tumors, and its overexpression was independent of imprinting status.

In conclusion, our findings support that LOI of IGF-II may be an epigenetic trait that is polymorphic in the population. We provide evidence that suggests that because LOI of IGF-II is associated with higher levels of IGF-II expression, it may play a role in the development of colorectal cancer. These findings are intriguing and need to be confirmed in larger studies. Furthermore, it will be important to assess whether additional genes that are imprinted, both within the 11p15.5 region and other imprinted cluster regions in the DNA of normal mucosal cells, can serve as predictors for adenoma occurrence.

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NOTES

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